AN ATTEMPT TO FIND A RAPID AND COST EFFECTIVE METHOD OF PROTEIN ISOLATION FROM HEPATIC TISSUE OF MAMMALS

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ABSTRACT: *The current study was aimed to design, a cost effective lysis buffer, which provides high Extractable Protein (EP) and compare its efficacy with routinely used buffers. In this study, four different buffer solutions i.e. I: phosphate buffered saline (0.1M PBS), II: sodium hydroxide (6N NaOH), III: sodium dodecyl sulphate (SDS-0.7%) and IV: (SDS-1.0%) with varying concentration and incubation time periods were examined. Liver tissues (1g) aliquots from mice were taken. For each extraction procedure, a set of 3 aliquots were used. Tissue homogenates were made for the respective buffers and their supernatants were used for Protein contents estimation using Bromocresol Green (BCG) method. The results revealed that all buffer used had significant effects on the levels of total protein extracted. Maximum EP contents were found with buffer-IV in 200µl and 45mins of incubation when compared to other procedures. Buffer-III also yields a good total protein but less than buffer-IV ($P<0.001$). It is concluded that though SDS is costly than the chemicals employed in other procedures but the quantity used is very minute and hence could be the choice for extraction from large sample number. This may help to maintain cost effectiveness of work. In addition, preparation of this lysis buffer is very easy and straight forward that can improves the efficiency of the whole process.*

Keywords: NaOH, PBS, Protein extraction and SDS.

INTRODUCTION

Proteins are the integral components of a cell and control vital physiological functions related to the cell proliferation, differentiation and death.A minor change in their structure affects its functions and expression which may lead to the development of disease [1,2,3].

Extraction of protein from mammalian tissue is challenging for scientists as the procedure is time consuming. It often involves large number of samples and tedious work that usually follows a series of steps of mechanical (liquid homogenization, french cell press, sonication, grinding with abrasives, agitation with glass beads, freeze/thaw etc.) and chemical nature (alkaline and detergent lysis) [4,5,6]. Lysis causes production of significant amount of heat and foaming which denature and degrade the surface, oxidation and generation of free amino acids. Amino acids like arginine, asparagine, glutamine, and serine could be completely destroyed while others are racemized. Thus more care and equipment is required to maintain the configuration, quality and original integrity of protein [7,8].

Detergents are organic compounds and mild surfactants, used for disruption of the cell membrane, cause release of the intracellular material in soluble form. They are crucial solubilizing agents for the isolation, purification, and crystallization of membrane proteins specifically [9]. Wide ranges of commercially available detergent are ionic, non-ionic and zwitterionic. Among these choices, ionic detergents (anionic or cationic) are able to perform cell lysis in seconds. Sodium dodecyl sulphate (SDS) is one of the ionic detergent and is able to perform cell lysis immediately [10].This anionic detergent is very effective surfactant in solubilizing almost all proteins. SDS binds to protein with a ratio 1:2 w/w (or one SDS anion per two amino acids) and therefore will mask the charge of the protein and will add a negative charge to all proteins in the sample despite of their isoelectric point (pI) [11]. Furthermore, SDS precipitates at low temperature, due to one of the highest Critical Micelar Temperature (CMT) among detergents. This is the main advantage and preferred in isolation of proteins [12]. The current work was aimed to optimize SDS as lysis buffer and compare its efficacy with routinely used buffers i.e. phosphate buffered saline (PBS), Sodium hydroxide (NaOH) so as to get high Extractable Protein (EP).

MATERIALS AND METHODS

Materials

All chemicals and reagents were of analytical grade and obtained from Sigma-Aldrich Chemie (Munich, Germany) or Merck (Darmstadt, Germany). Kits from Randox Laboratories, Ltd (U.K) were used for the estimation of protein and albumin activities.

Experimental design

Livers of six normal albino mice were excised, as described elsewhere [13] and processed for protein extraction and quantification to compare four different buffers with varying concentration and incubation time periods for their maximum yield (Table I).

Protein extraction protocols

Liver tissues from six controlled previously dissected mice were taken and equal weight (1g) aliquots were made. By using a tissue homogenizer the tissues were homogenized in the respective buffer to obtain the homogenate sample of crude total protein. Homogenates were centrifuged for 20 minutes at 2000×g to clarify samples and to provide better compression of the pellet. The samples were not heated upon extraction. Centrifugation was repeated to improve separation from lipids and the pellet. The supernatants were used for the total protein and albumin estimation according to the manufacturer's instructions.

Incubation conditions

For buffer-I and II, tissue aliquots was allowed to dissolve in 100ml of buffers with incubation temperature of 25°C for 24h and 40°C for 48h, respectively while for buffer-III and IV, 200µl and 1000µl of buffers were added in tissue aliquots separately. These homogenates were then incubated at different time periods (15, 30 and 45min) for both buffers each.

Statistical analysis

The data were analyzed using Prism Graph pad 5 software (San Diego, CA). Statistical significance was calculated by one-way analysis of variance (ANOVA) and Tukey post test. Significance was accepted at $P < 0.05$. Results are shown as Mean ±S.E.M. with n=6.

RESULTS AND DISCUSSION

To date many methods have been developed and reported, but there is no single extraction method exists that can be applied universally for all kind of tissues. The purpose of the current study was to compare, different lysis buffer with varying dilutions and concentrations. These buffers were compared in extraction of maximum protein yield from hepatic tissue of mice. Extraction in these experiments was assessed by determining the amount of total Extractable Protein (EP) or the protein that could be released from a tissue.

Maximum yield of total protein and albumin was noted using buffer-IV and III (8.60& 5.70 g/dl; 7.20 & 4.00g/dl) compared to buffer-I ($P<0.0001$), while buffer-II showed low protein yield as compared to buffer-IV (Table I). The low yield in buffer I&II might be due to the fact that some proteins require harsher conditions for efficient transfer to the solubilized state. The ingredients in these buffers have a very high buffering capacity and are highly soluble in water, which used to disengage attached and clumped cells [14,15]. Based on these findings, extraction buffer-III and IV were selected and further optimized.

In buffer-III, total proteins and albumin concentration was found to be maximum for 1000µl (7.2 & 4.0g/dl) and 200µl (7.86 & 3.53g/dl) of dilutions after 30 minutes of incubation, compared to 15 and 45min. Regarding buffer-IV, these parameters were found to be maximum after 15minutes of incubation, for 1000µl (2.6 & 1.7g/dl) and 200µl (8.10 & 5.60g/dl) of dilutions compared to 30 and 45min (Table II). The best possible yield of protein was obtained with SDS. Experimental set of 1% SDS in 200µl and 45min of incubation at 37°C, showed the highest amount of total protein and albumin contents (8.60 & 5.7g/dl) suggesting that this protocol was successful. Bhaduri and Demchick [16] employed two ingredients (acetone-SDS mixture) for extraction of proteins from bacterial cells. However, no significant amount was able to be extracted from bacterial cells. On the contrary, in current study SDS alone worked well in rupturing the cell membrane and extracted protein immediately. This might be due to the fact that SDS being a strong ionic detergent is able to lyse cells

Table 1: Comparison of four different protein extraction buffers for their for maximum Protein yield.

Lysis Buffer (Composition &Concentration)	Total Volume (ml)	Incubation period (H)	Incubation Temperature (°C)	TotalProtein (g/dl) ±S.E.M.	Albumin (g/dl) ±S.E.M.	Globulin (g/dl) ±S.E.M.	A/G Ratio ±S.E.M.
I (PBS-0.1M)	100	48	25	5.400[b] ±0.11	1.933[c] ±0.14	3.467[b] ±0.14	0.500 ±0.05
II (NaOH-6N)	100	24	40	6.333[b] ±0.21	3.267[c] ±0.24	3.067 ±0.08	1.033 ±0.08
III (SDS-0.7%)	1	0.5	37	7.200[b,c] ±0.03	4.000[c] ±0.11	4.000 ±0.11	1.267 ±0.06
IV (SDS-1.0%)	0.2	0.75	37	8.600[b,c] ±0.11	5.700[c] ±0.11	2.900[b] ±0.01	1.967 ±0.03

Abbreviation used: A/G=Albumin/Globulin.
([a]P<0.05, [b]P<0.01, [c]P<0.001)

Table II: Optimization of concentrations, volumes and incubation period, using buffer-III (SDS 0.7%) and IV (SDS 1.0%).

Dilution (µl)	Incubation period (min)	Total Protein (g/dl) ±S.E.M.	Albumin (g/dl) ±S.E.M.	Globulin (g/dl) ±S.E.M.	A/G Ratio ±S.E.M.
1000	**15**	6.767±0.03 (2.667±0.03)	3.933±0.06 (1.700±0.11)	2.733±0.11 (0.733±0.06)	1.200±0.11 (2.400±0.11)
	30	7.200±0.03 (2.500±0.05)	4.000±0.11 (1.633±0.06)	3.200±0.10 (0.600±0.05)	1.267±0.06 (2.200±0.11)
	45	6.633±0.17 (2.267±0.03)	3.900±0.11 (1.633±0.06)	2.800±0.06 (0.533±0.06)	1.167±0.08 (2.600±0.11)
200	**15**	2.700±0.05 (8.100±0.05)	2.333±0.06 (5.600±0.11)	0.333±0.06 (2.667±0.06)	5.800±0.11 (2.133±0.06)
	30	7.867±0.03 (8.067±0.03)	3.533±0.14 (4.500±0.11)	4.200±0.11 (3.400±0.11)	0.733±0.06 (1.400±0.11)
	45	4.067±0.03 (8.600±0.01)	3.167±0.08 (5.700±0.11)	0.800±0.11 (2.900±0.00)	3.967±0.08 (1.967±0.03)

An abbreviation used: A/G=Albumin/Globulin.
Values in the parentheses indicate the corresponding values found for Buffer-IV.

and then leaches out large majority of total proteins.

In this study, a novel protein extraction procedure, which is at the same time cost-effective and time-saving for obtaining proteins from hepatic tissues of mice has been successfully developed and optimized that combines several desirable aspects. Proteins are extracted very rapidly after 45min of incubation in 200µl of 1.0% SDS. Thus, lysis buffer-IV offers rapid and cheaper protocol, which may be applicable to routine laboratory protein estimate analysis.

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